

Anti blood group-M autoantibodies with livedo reticularis, Raynaud's phenomenon, and anaemia

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SUMMARY A 63-year-old woman presented with Raynaud's phenomenon and extensive cold-induced livedo reticularis. A skin biopsy showed no abnormality of the blood vessels but the blood contained high titres of a very unusual autoantibody against the M blood group, most active at low temperatures. An IgM cryoglobulin was detected, and anti-M activity was found in this fraction. The cells of the patient were grouped as MM. The direct antiglobulin test was positive due to C₃ component of complement bound to the red cells. The haematological and biochemical results indicate a mild haemolytic process, which is at present well compensated.

Livedo reticularis may occur in a wide variety of diseases affecting the blood vessels of the skin or the rheological properties of the blood (Champion, 1965; Copeman, 1975). We describe the case of a woman with Raynaud's phenomenon and severe and extensive livedo reticularis regularly induced by cold and abolished by warmth, in whom the only abnormality found was an unusual anti-M autoantibody most active at low temperatures.

Case history

A 63-year-old housewife was admitted to The London Hospital in May 1977 complaining of purplish discoloration of the limbs. Ten months before admission she developed severe intermittent cramping pain in the upper arms, which was worse at night; this lasted for about three months and then resolved. At about the same time as the pain she noticed purple mottling especially of the hands but also of the arms and legs. This was worse in the cold and disappeared completely when she was very warm. For six months before admission she had noticed Raynaud's phenomenon affecting the hands. Prednisolone, 10 mg daily, had been given for some weeks without benefit. She had had tuberculosis of the spine and rheumatic fever as a girl. She had had two miscarriages, and two live births with healthy infants. She had never had a blood transfusion. For some years before admission

she had been taking piperazine oestrone sulphate, 3 mg daily, for postmenopausal flushing. On examination she was a little pale. When she was cool there was livedo reticularis over the arms and legs with no palpable nodules or ulceration. This disappeared when she was warm in a hospital bed. The peripheral pulses were normal and there were no other abnormal physical signs.

INVESTIGATIONS

Haemoglobin 9.5 g/dl, haematocrit 0.291, mean corpuscular volume 92 fl, mean corpuscular haemoglobin 30.3 pg, mean corpuscular haemoglobin concentration 32.4 g/dl. Anisocytosis, polychromasia, and agglutination of the red cells were noted, but unfortunately a reticulocyte count was not performed. The blood count was repeated three months later in warm weather and showed a haemoglobin of 12.8 g/dl and a reticulocyte count of 1.2%. The white cells and platelets were normal. Serum total bilirubin 28 μ mol/l (1.6 mg/100 ml) direct 5 μ mol/l (0.3 mg/100 ml), liver function tests otherwise normal. A biopsy of affected skin showed no abnormality on histological or immunofluorescent examination; the blood vessels were normal. The following were also normal or negative: antinuclear factor and auto-antibody screen, latex fixation test, plasma protein electrophoresis, serum iron, vitamin B₁₂ and folate, red-cell folate, urea and electrolytes, Treponema pallidum, haemagglutination, and VDRL slide test, microscopy and culture of the urine, and x-rays of the chest, skull, and hands. X-ray of the lumbar

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spine showed partial fusion of the fourth and fifth vertebrae but no sign of active tuberculosis.

Special investigations

METHODS AND MATERIAL

Phosphate buffered saline (PBS)

0.15 M saline in Sørensen's phosphate buffer, pH range 6.0 to 8.0.

Direct antiglobulin test (DAGT)

0.2 ml of cells are washed four times in PBS pH 7.2 and tested against specific antihuman globulin IgG, anti C3/C3c, anti-IgM, anti-IgA (Behring Werke AG) by the standard tile technique, and read after 5 minutes.

In the following testing procedures agglutination reactions were read microscopically at the appropriate temperatures of incubation (4°C, 17°C, 30°C, and 37°C) using the standard scoring system + + +, + +, +, ±, -.

Saline reactions

1 vol 3% suspension of cells in PBS pH 7.2 was mixed with 1 vol of serum and read microscopically after 1½ hours' incubation.

Papain reactions

1 vol of serum was mixed with 1 vol of 5% suspension of papain-treated cells PBS pH 7.2 (Mollison, 1972), and agglutination was assessed macroscopically after one hour's incubation.

Albumin displacement

1 vol of serum was mixed with 1 vol of 3% cells in PBS pH 7.2 after 1½ hours, 1 vol of 20% serum albumin (30% albumin diluted in AB serum to 20%) was added, and agglutination was read microscopically after a further 30 minutes' incubation.

Indirect antiglobulin test

Automatic Coombs washer (Spectra-Auto II) with AHG reagent incorporated in the cycle was used. 4 vol serum + 1 vol 20% suspension of cells in saline PBS pH 7.2 were incubated for one hour at 37°C, before washing four times in the machine. After completion of the cycle the residuum was placed on a tile for macroscopic examination of agglutination and performance of the wash control.

Titration

All sera were titrated by master-dilution technique using Pasteur pipettes and calibrated for the volumes required, with careful technique to avoid 'carry-over'. The agglutination was read microscopically.

Cryoglobulin measurement

This was carried out by the method of Weisman and Zvaifler (1975).

Results

The direct antiglobulin test was found to be weakly positive owing to the presence of complement C₃ bound to the red cells. A strong anti-M antibody was detected in the serum reacting over a wide thermal range of 4°C to 37°C. Up to a temperature of 17°C the antibody reacted with MM, MN, and NN cells but only with MM and MN cells at 30°C and above, as shown in Table 1. It reacted with the patient's own cells at all temperatures, and the patient was typed as MM, giving negative results with several rabbit anti-N sera and an extract of *Vicia graminea*.

Table 2 shows the titration results of the anti-M over a wide thermal range. Albumin addition techniques as well as saline were used, as cold agglutinin titres correlate better with the degree of haemolytic anaemia in the presence of bovine albumin than in saline (Garratty *et al.*, 1977). Reactivity of the anti-M

Table 1 Specificity of the autoantibody over a wide thermal range

	O Adult cells							Own cells	O cord	
	MM	MM	MN	MN	MN	NN	MM	MN	MM	NN
4°C Saline	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +
4°C Papain	0	0	0	0	0	0	0	0	0	0
17°C Saline	3 +	3 +	2 +	2 +	2 +	1 +	3 +	2 +	3 +	1 +
30°C Saline	3 +	3 +	2 +	2 +	2 +	0	3 +	2 +	3 +	0
30°C Albumin	3 +	3 +	2 +	2 +	2 +	0	3 +	2 +	3 +	0
37°C Saline	1 +	1 +	1 +	1 +	1 +	0	1 +	1 +	1 +	0
37°C Albumin	3 +	2 +	2 +	2 +	2 +	0	3 +	2 +	3 +	0
37°C Papain	0	0	0	0	0	0	0	0	0	0
37°C IAGT	1 +	1 +	0	0	0	0	1 +	0	1 +	0

The antibody reacted equally with 0 MM and 0 NN cells at temperatures of 17°C and below but showed auto anti-M specificity at 30°C and above. Non reactivity with papain-premodified adult cells at 4°C excludes the presence of anti I.

Table 2 Titration results over a wide thermal range with adult, cord, and patient's own cells

Temp. (°C)	Titres with red cells							
	Adult red cells						Cord red cells	
	O MM Sal	O MM Alb	O MM Pap	o/c Sal	o/c Alb	o/c Pap	O MM Sal	O MM Alb
4	2000	2000	1	2000	2000	1	2000	2000
17	1000	1000	1	2000	2000	1	1000	1000
30	32	64	0	32	32	0	32	64
37	1	8	0	1	4	0	0	8

o/c = patient's own cells

Sal = buffered saline pH 7.2

Alb = albumin-addition technique using 20% serum albumin

Cells and serum were pre-incubated at the appropriate temperatures before mixing.

These results show that the reactivity of the anti-M was enhanced by the use of albumin-addition at 37°C.

Table 3 Anti-M activity tested at 30°C and 37°C at pH range 6.0 to 8.0

Temp (°C)	pH Saline	Titres with adult red cells			
		O NN	O MM	O MN	o/c
30	6.0	0	32	32	64
	7.0	0	32	16	32
	8.0	0	1	4	4
37	6.0	0	32	32	32
	7.0	0	1	1	1
	8.0	0	0	0	0

o/c = patient's own cells

The activity of the anti-M here shows characteristic pH dependence.

was increased at temperatures above 30°C in the presence of albumin.

The antibody showed typical dosage characteristics of anti-M, that is, variation in strengths of reaction with homozygous and heterozygous cells for the M antigen. It was not active with papain-treated cells. At 30°C and 37°C its activity was pH dependent (Table 3).

INVESTIGATION OF SERUM PROTEINS

Serum immunoglobulins were assayed using the Mancini technique yielding the following results:

IgG 15.28 g/l (normal range 5.00-15.0 g/l)

IgM 1.25 g/l (normal range 0.60-1.80 g/l)

IgA 3.36 g/l (normal range 0.75-2.50 g/l).

No paraprotein band was demonstrated on cellulose acetate electrophoresis, and no abnormal arcs were detected by immunoelectrophoresis. The serum used in these tests was collected and separated at 37°C. All tests were performed at 37°C. The serum cryoglobulin was 0.39 mg/ml. The normal range in this laboratory is 0.0-15 mg/ml

The washed cryoprecipitate was tested by the Mancini technique for IgG, IgM, IgA, and C₃ and by Ouchterlony and immunoelectrophoresis for the presence of IgG, IgM, IgA, C₃, and fibrinogen. The IgM level in the cryoprecipitate was estimated to be

0.27 mg/ml. No IgG, IgA, or C₃ was detected. Both kappa and lambda chains were detected by Ouchterlony and immunoelectrophoresis, and the anti-human whole serum gave only one arc identifying with anti-human IgM serum.

The cryoprecipitate fraction prepared was tested against cells in PBS pH 7.2, which indicates that it possesses anti-blood group M activity (Table 4).

It is intended to continue our investigations of the cryoglobulins and to look for the presence of immune complexes.

Table 4 Agglutination of cryoprecipitate fraction in PBS

	O MM	O NN	O MN
Saline 17°C	3 +	0	2 +

Discussion

Livedo reticularis is a physical sign seen wherever the flow of blood in the subpapillary venous plexus of the skin becomes so slow that the vessels dilate and fill with deoxygenated blood. This forms a bluish network. This slowing of blood in the subpapillary plexus is seen in healthy subjects in the cold and also in various diseases, as a result of either arterial damage or the presence of abnormal constituents of the blood, which either block the arteries or alter the flow properties of the blood passing through the capillaries and venules (Champion, 1965; Copeman, 1975). Slowing of blood in the small vessels of the conjunctivae is observed in patients with cold agglutinin disease when ice is applied, and flow returns to normal on warming (Iwai and Mei-Sai, 1925; Nelson and Marshall, 1953). Most patients with cold-agglutinin disease show Raynaud's phenomenon, cyanosis, livedo reticularis, and ischaemia of those parts of the body most exposed to the cold: the hands, nose ears, and toes (Ferriman *et al.*, 1951; Dacie, 1962). Similar changes may be provoked on

other parts of the body on cooling (Schubothé, 1966).

Although our patient's livedo reticularis was much more prominent in the cold and disappeared on thorough warming, it is not clear why she should have such extensive livedo reticularis. This could reflect the wide thermal range of her antibody, but it is more likely to be due to the association of her antibody with cryoglobulins, which can be associated with extensive livedo reticularis (Mackenzie *et al.*, 1961).

Anti-M autoantibodies appear to be very rare. Bowes (1976) reported a case of a woman with Raynaud's phenomenon, anaemia, and anti-M autoantibody but did not mention whether cryoglobulins were present. Other examples of auto-anti-M have been reported in the literature (Fletcher and Zmijewski, 1970; Hysell *et al.*, 1973; Tegoli *et al.*, 1970) but the majority of cases did not show any similar clinical significance. Other blood group specificities have been implicated with cold-agglutinin disease; these are anti I, anti i (Issitt and Issitt, 1976), anti-Gd, and anti-Pr (Roelcke *et al.*, 1976). These are rarely associated with cryoglobulins.

We have not treated our patient so far because her symptoms are relatively mild, but plasmapheresis and continuous-flow centrifugation (Taft *et al.*, 1977) and chlorambucil treatment may have to be considered. There is no evidence of lymphoma or other malignant disease in this patient. However, Schubothé (1966) notes that there is a steady increase of lymphoid cells in the bone marrow of many patients with cold-agglutinin disease and suggests that it may represent a chronic lymphoproliferative disorder.

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